Introduction

Problem, Rationale, and Significance

In the United States, an average of 229.2 million metric tons of organic municipal solid waste (i.e. yard wastes, soiled paper and paper products, food and food processing wastes, organic industrial wastes, and woody waste) (USEPA, 2005), eight million dry metric tons of sewage sludge (USEPA, 1999), and six million dry metric tons of manure from confined animal farming operations (Economic Research Service, 2001) are generated each year. Safe handling, processing, and disposal of organic wastes involve the consideration of social, economic, and environmental costs. Applying these materials to land is a desirable means of disposal because it decreases total disposal costs, reduces the amount of material added to landfills, and recycles nutrients in soil systems (USEPA, 1999). Raw organic wastes are typically bulky and may have high pathogen levels and vector attraction. A process often employed to treat such wastes prior to land application is composting, which is the controlled, thermophilic, partial degradation of organic matter into a humus-like product (Adoni et al., 1999). Composted wastes have reduced volume, mass, vector attraction, pathogen content and weed seed viability than non-composted wastes. These benefits greatly increase the value of compost as a soil conditioner (Epstein, 1997).

The composting process significantly changes the physiochemical properties (e.g., bulk density, pH, nitrogen:phosphorus ratio, total carbon, carbon:nitrogen ratio, total nitrogen content, and plant available nitrogen) of organic wastes. Observed decreases in compost total nitrogen (Eneji et al., 2000; Nishio and Oka, 2003; Lin et al., 2003; Guster et al., 2005) are due to ammonia volatilization from microbial metabolic processes, the incorporation of nitrogen into the microbial biomass, and the fixation of nitrogen into humic substances, the major constituents of "stable" organic matter (Epstein, 1997).

Variability in starting materials and composting conditions influence the final composition and biological, physical, and chemical attributes of compost. Determining proper application rates that satisfy crop nutrient needs and minimize environmental degradation is essential to sound field management. Compost applications may be based on estimated plant available nitrogen contents; however, predicting compost nitrogen availability is difficult because nitrogen is slowly made available to plants via organic
matter mineralization (Glenn, 1992). Plant available nitrogen consists of the sum of inorganic N not lost via ammonia volatilization and the fraction of organic N that mineralizes to inorganic N during the season of application. A commonly employed method for estimating plant available nitrogen of various organic wastes is the use of greenhouse studies that compare plant N uptake from known rates of inorganic N to known rates of organic residuals containing known amounts of inorganic and organic N. In such studies, the relative nitrogen fertilizer equivalency of compost is compared to inorganic fertilizer (Muñoz et al., 2004).

Researchers have observed that the nitrogen content of organically amended soils is higher than soils receiving inorganic fertilization (Singh and Ghosh, 1999; Ceuvas et al., 2000; Chang and Cheng, 2000; Khatik and Dikshit, 2001; Sullivan et al., 2003; Rodriguez et al., 2005). This is likely due to higher rates of total nitrogen applied in organic wastes than in inorganic fertilizers in order to meet plant nutrient needs from the incompletely plant available nitrogen forms in such residuals.

Researchers have also observed that composted organic amendments can improve plant health beyond the nitrogen fertility value (Ayuso et al., 1996; Vadrighi et al., 1996; Buckerfield et al., 1999; Atiyeh et al., 2000a; Atiyeh et al., 2000b; Atiyeh et al., 2001; Atiyeh et al., 2002). Of particular interest is the apparent ameliorating effect of organic amendments on drought-stressed crops. Sahs and Lesoing (1985) observed higher sweet corn yields in plots amended with beef feedlot manure than those that were inorganically fertilized during drought years. Hornick (1988) also observed increased sweet corn yields in organically amended sand and gravel spoils relative to plants that received inorganic fertilizer. HuiLan et al. (1998) noted that the application of organic amendments increased water stress resistance of sweet corn leaves. In particular, stomatal and curricular conductances of the leaves were lower in these plants than in inorganically-fertilized plants. In five-week old water stressed maize seedlings, Xu (2000) recorded higher photosynthetic rates when the soils were organically amended. Heckman et al. (1987) found that field grown soybeans fertilized with sewage sludge had increased drought resistance and nitrogen fixation than the control treatment.

The phytohormonal activity of humic substances in composted organic amendments is thought to play a causal role in drought stress amelioration (Xu, 2000).
Subler et al. (1998) argue that the presence of humic substances may enhance crop production through directly providing plant growth regulators (phytohormones) or by stimulating microbial activity that supplies the substrates for hormone synthesis. Fossil-derived humic substances have been foliarly applied in most drought stress studies to investigate potential differences in plant physiology and metabolism (Xudan, 1986; Zhang and Schmidt, 1999; Zhang and Schmidt, 2000; Zhang and Ervin, 2004). There have been very few studies that investigated effects of land applied compost on leaf health, yield, and yield quality of drought stressed agronomic crops.

**Thesis Objectives**

1. Calculate nitrogen mineralization rates of composted and non-composted organic materials applied to a Fauquier silty clay loam in a greenhouse pot study in order to assess the nitrogen contributions to plant response in a corresponding field study. I hypothesize that organic amendments with a lower C:N ratio will mineralize more nitrogen than amendments with a greater C:N.

2. Determine the changes in soil physiochemical properties (e.g., nutrient concentrations, bulk density, water holding capacity, organic carbon, humic and fulvic acid-carbon) in a long term compost field study conducted on a Fauquier silty clay loam. I hypothesize that the Panorama yard waste compost, Rivanna biosolids compost, and poultry litter amendments applied at 100% agronomic N rates will increase soil fertility, organic and humic carbon content, decrease bulk density, and increase water holding capacity more than the same amendments applied at 30% agronomic N rates and the inorganically fertilized and control treatments.

3. Determine corn and soybean total leaf protein and chlorophyll contents; leaf water potential and photochemical efficiencies; leaf antioxidant activities (i.e., superoxide dismutase, ascorbate peroxidase, catalase); lipid peroxidation (i.e. malondiadehyde concentration), air temperature-leaf temperature (Delta T) (soybean only); yield and seed quality (i.e., protein, oil, starch, density, and fiber (soybean only)) during the summer 2004 and 2005 field seasons. I hypothesize that crops grown in the organically amended treatments (see objective # 2) applied at 100% agronomic N rates will have equal or greater leaf protein and
chlorophyll contents, greater antioxidant activity, lower malondiadehyde concentrations and Delta T values, and increased yields and seed quality than the same amendments applied at 30% agronomic N and the inorganically fertilized and control treatments.

**Previous Work**

*Compost May Increase Nutrient Availability*

**Nitrogen**

Nitrogen mineralization is controlled by compost properties including organic carbon content, C:N, total nitrogen, and plant available nitrogen; soil moisture; microbial activity; and soil texture (Eghball et al., 2002; Agehara and Warnke, 2005; Cabrera et al., 2005). Compost C:N greater than 25:1 have been shown to immobilize nitrogen in soils (Tester et al., 1982; Chen et al., 1996; Douglas et al., 2003; Nishio and Oka, 2003; Gagon et al., 2004; Flavel et al., 2005). Although often used to estimate nitrogen mineralization (Sikora and Yakovchenko, 1996; Kumar and Goh, 2003; Khalil et al., 2005), some researchers concluded that C:N did not accurately estimate nitrogen mineralization and stated that total nitrogen was a better estimator of nitrogen mineralization (Kuo, 1995; Kessel and Reeves, 2003; Cabrera et al., 2005).

Low soil moisture conditions can decrease nitrogen mineralization. Eghball (2002) observed greater soil NO$_3^-$ contents in inorganically fertilized soils than organically amended soils when precipitation was 56% and 80% below average. He concluded that this was due to decreased microbial activity in the amended soils. Many researchers have observed decreased nitrogen mineralization in fine- to medium- textured soils relative to course-textured soils (Veen et al., 1985; Sims, 1990; Benitez et al., 1997; Omay et al., 1997; Egelkraut et al., 2000; Griffin et al., 2002; Preusch et al., 2002), though a few have not (Ladd et al., 1977; Motavalli et al., 1989; Giardina et al., 2001). Thomsen and Olesen (2000) observed no differences in nitrogen mineralization based on soil type in a 266 day incubation study. Sørensen and Jensen (1995) observed that the protective capacity of finer soil particles (i.e. clay minerals) decreases over time. Finer textured soils may temporarily limit nitrogen mineralization due to physical protection of organic matter, the presence of anaerobic microsites within aggregates, and/or the greater
The larger pore sizes of coarse-textured soils renders recently mineralized nitrogen more susceptible to plant uptake or leaching (Nardi et al., 2004). Indeed, Davis et al. (1997) and Badran et al. (2000) observed less residual NO$_3^-$ in sandy versus clayey soils.

**Phosphorus**

The application of organic amendments to soils has been shown to increase soil phosphorus content and plant available phosphorus (Mugwira, 1979; Lund and Doss, 1980; Reddy et al., 1980; Field et al., 1985; Schlegel, 1992; Kingery et al., 1994; Gao and Chang, 1996; Clark et al., 1998; Eghball et al., 2004). The N:P ratio of composted materials is relatively low due to nitrogen losses during composting (Eghball, 2002; Luo et al., 2005; Rodriguez et al., 2005). Compost applied on a nitrogen basis may significantly increase soil phosphorus levels as this ratio is usually significantly lower than crop N:P uptake (Kuo, 1995; Eghball et al., 2002; Park et al., 2004; Singer et al., 2004). Eghball et al. (2004) observed continued plant phosphorus uptake up to four years after nitrogen-based compost and manure applications ceased without the addition of phosphorus fertilizers. Excessive soil phosphorus levels are not detrimental to plant health, but can cause serious environmental degradation if the soil is subject to runoff and erosion.

**Potassium, Secondary and Micronutrients**

Compost may contain appreciable levels of potassium, calcium, magnesium, and micronutrients. The presence of these elements is usually due to feedstock composition and pre-composting processing. For example, biosolids are sometimes stabilized or dewatered with calcium hydroxide (Faulker, 2001), which can greatly increase the calcium content. Animal feed is often supplemented with minerals (e.g., copper, zinc, manganese) to maximize animal growth and development (Blezinger, 2001; Kegley, 2001). The ability of animals to utilize these minerals is generally less than the amount of mineral provided in the feed. It is consequently excreted as waste and incorporated into the organic amendment matrix (Kingery et al., 1994). Numerous researchers have documented increases in soil potassium (Kumar and Kumaraswary, 2000; Khatik and Dikshit, 2001; Chang and Cheng, 2000; Rodriguez et al., 2005) calcium and magnesium.
Abreu Junior et al., 2001; Eghball et al., 2002; Walker and Bernal, 2004), and micronutrients (Clark et al., 1998; Bulluck et al., 2002; Negm and Zaki, 2004; Yogananda et al., 2004) after the addition of composted materials.

The Humification of Compost

Nutrient mineralization in compost is attributed to the "active" carbon pool, where carbon is easily mineralized by microbes and serves as an available nutrient source to plants and microorganisms. The relative stability of compost (i.e. its resistance to rapid mineralization) is due to the presence of a "stable" carbon pool, where decomposed biomass is in a humified semi-final state. Humification occurs during composting and is the transformation of organic material into high molecular weight molecules termed humic substances (Adoni et al., 1999).

Humic Substances Defined

Humic substances are the major constituents of stable organic matter. These materials are naturally occurring, ubiquitous organic compounds that contain relatively high molecular weights, are yellow-black in color, and are formed by secondary synthesis reactions between plant and animal remains and microbial metabolites (Stevenson, 1994b). Humic substances are operationally defined, based on solubility, into three classes: fulvic acid, humic acid, and humin. Fulvic acids represent about 20% of humic substances (Epstein, 1997), are relatively low in molecular weight (1000-4000 g/mol), and soluble in both alkali and acidic solutions (Stevenson, 1994b). Humic acids represent roughly 80% of humic substances (Epstein, 1997), have relatively large molecular weights (12,000-300,000 g/mol), and are insoluble in acidic solutions (Stevenson, 1994b). Humin is the non-extractable humic acid fraction. It is defined as very large humic acid molecules that are tightly bound to minerals and cannot be extracted (Stevenson, 1994a).

There are three major accepted theories of humic substances synthesis: the lignin theory, the polyphenol theory, and the sugar-amine theory. The lignin theory, popularized by Waksman (1932) is based on the assumption that humic substances represent modified lignins. Because lignins are resistant to microbial decay and are major plant polymers, they are often considered a major contributor to humic substances synthesis. Lignins oxidized by microbial degradation undergo condensation reactions with microbial amino
acids to yield the humic molecule. Waksman (1932) cites the following as evidence to support the lignin theory:

i.) Both lignin and humic acid are decomposed with considerable difficulty by the great majority of fungi and bacteria.

ii.) Both lignin and humic acid are soluble in alkali and precipitated by acids. Both are partly soluble in alcohol and pyridine and contain $\text{OCH}_3$; groups whose content diminishes during decomposition.

iii.) Both lignin and humic acid are acidic in nature, both are able to combine with bases, and both are characterized by their capacity to undergo base exchange, although to a different quantitative extent.

iv.) When lignins are warmed with aqueous alkali, they are transformed into methoxyl-containing oxidized humic acid. Humic acids have many properties in common with oxidized lignins.

The theory that degradation of complex polymers yields humic substances supports the assumption that larger humic molecules (i.e., humic acid) are synthesized first, and that their degradation yields smaller humic substances (i.e. fulvic acid).

The tenets of the polyphenol theory state that under aerobic conditions, lignin may be broken down into monomeric structural units such as polyphenols and quinones. These molecules react with microbially synthesized quinines and amino acids to form fulvic acid. Polymerization of several fulvic acids yield one humic acid molecule. Further polymerization of humic acid yields humin (Stevenson, 1994b).

The third theory of humic substances formation is the sugar-amino condensation theory (Stevenson, 1994b), also known as the melanoidin pathway and the Maillard reaction. Sugars and amines, which are are relatively abundant in the soil environment, condense to form fulvic acid. Further polymerizations yields humic acid and finally humin.

Within the soil microenvironment, there is simultaneous microbial synthesis and degradation of newly formed humic substances. This likely prevents polymerization and the synthesis of larger humic acid molecules. Fulvic acid synthesis, therefore, is more likely explained by the latter two theories, and humic acid synthesis is more likely explained by the lignin theory.
Compost and Soil Humic Substances

The humification of composted organic wastes is much lower than that of native soil organic matter due to the relatively short composting period (Valasco et al., 2004). Researchers have observed the development of humic-like materials in composted materials over time. Tiquila (2003) observed an increase in fulvic and humic acid-like substances, and an increase in HA:FA of composted pig litter. Baddi et al. (2003) observed an increase in fulvic and humic acid-like substances, an increase in aromatic carbon, and a decrease in aliphatic carbon over time in composted olive mill waste over time. Chefetz et al. (1998) also observed an increase in aromatic carbon in composted municipal solid wastes over time, indicative of the increased stability of the final product. Numerous researchers have observed that humic-like substances extracted from composts are similar to, but poorly developed relative to soil organic matter. Fluorescence and electron spin spectroscopy reveal that these humic-like substances contain a greater nitrogen content (Boyd and Sommers, 1990; Ouatmane et al., 2002; García-Gil et al., 2004; Senesi, 2004), are more aliphatic (i.e. less aromatic) (Giusquiani et al., 1994; Yakimenko et al., 1995; Yona, 2003; Brunetti et al., 2005), and are more heterogenous in composition than native soil organic matter (Unsal and Ok, 2001; Senesi, 2004; Brunetti et al., 2005).

The humic-like substances of composts in amended soils have been documented to approach molecular properties typical of native humic substances (Senesi, 2004). There is a growing consensus that the humic-like structures of composts can be incorporated into native soil humic substances (Tomati et al., 2001; Gigliotti et al., 2003). Indeed, Leifeld et al. (2002) observed that the decomposition of sewage sludge compost in soil was the ongoing humification process of the compost itself.

The accumulation of humic substances ensures long-term maintenance of soil organic carbon (Xiang et al. 1998; Zinati et al. 2001; Dorado et al. 2003; Rivero et al., 2004). Researchers have observed increases in soil organic matter content in gravelly (Hornick, 1988; Zinati et al., 2001), sandy (Foley and Cooperband, 2002; Negm and Zaki, 2004), silty (Borken et al., 2002; Tsegaye et al., 2003), and clayey (Rivero et al., 2004; Yuksel, 2004) soils following compost applications. Filip and Kubát (2001) concluded that increased soil organic matter content observed in long-term organically
amended soils could be attributed to the increased resistance of humic acid fractions to microbial degradation.

**Compost May Increase Soil Water Holding Capacity**

A reduction in soil water holding capacity is considered by some to be the major factor contributing to reduced yields in eroded, low organic matter soils (Bauer and Black, 1992). The addition of organic amendments has been documented to increase soil water holding capacity (Hernando et al., 1989; Shiralipour et al., 1992; Curtis and Claassen, 2005; Konomi et al., 2005). Soil water holding capacity is operationally defined as water held at specific tensions (i.e., “field capacity” or “wilting point”) or the total amount of water held between two tensions. It is often considered an indirect measurement of plant available water. However, a plant's ability to extract water from a particular soil is also species dependent. An increase in carbon decreases soil bulk density through increased aggregation of soil particles and the relative number of smaller pores (Khaleel et al., 1981; Martens and Frankenberger, 1992; Warren and Fonteno, 1993; Canarutto et al., 1996; Grandy et al., 2002; Elsharawy et al., 2003). This shift in pore size distribution (i.e. smaller pores), increases the tension required for pore drainage (Khaleel et al., 1981). Humic substances in organic amendments have been documented to bind soil microaggregates (Elsharawy et al., 2003).

The greatest improvements in soil water holding capacity have been documented in the coarse-textured soils (Abdel-Sabour et al., 1997; Stepkowska et al., 2001; Foley and Cooperland, 2002; Hangarge et al., 2002; Elsharawy et al., 2003; Newman et al., 2005). Researchers have observed that the addition of organic material increases the number of smaller pores and surface area of sandy soils; thus, more water is held in amended soils at lower tensions. The amount of water retained in organically-amended soils is greater than that retained in unamended soils as soil-water tension increases (Khaleel et al., 1981; Elsharawy et al., 2003), and the overall water holding capacity of the soil is increased. Improvements in soil water holding capacity with the addition of organic amendments are typically less significant or simply not observed in finer-textured soils (Khaleel et al., 1981; Reynolds et al., 2003; Jiménez et al., 2004) although researchers have observed increases in water holding capacity at very high application rates (Sheng-Gao and Lei, 2004). The addition of organic matter in finer textured soils
results in a slight increase in moisture holding capacity at low tensions (i.e., field capacity) and no change at higher tensions (i.e., wilting point) (Khaleel et al., 1981; Carter et al., 2004). Nearly all pores are air filled at higher tensions, and the moisture content is determined by the specific surface area of soil particles and the thickness of water films (Khaleel et al., 1981). Finer soil particles have a very large surface area. The addition of organic amendments, therefore, does not improve water holding capacity at higher tensions.

Pooling data from several studies, Khaleel et al. (1981) observed that 80% of the variability in water holding capacity at low tensions (i.e 0.03 MPa) and high tensions (i.e. 1.5 MPa) could be explained by soil texture and organic carbon content. Bauer and Black (1992) observed that variations in percent water holding capacity of sandy, medium, and fine textured soils could be based on sand content with 75% certainty.

Although increases in soil water capacity cannot be directly associated with increased crop yield, it is generally accepted that increases in water holding capacity between 0.03 and 1.5 MPa is beneficial to crop growth. Increased “plant water availability” has been considered the causal factor for increased crop yields in organically amended soils (Serra-Wittling et al., 1996; Hangare et al., 2002; Curtis and Claassen, 2005). Interestingly, some researchers have demonstrated that organic amendments may produce an ameliorating effect on drought stressed crops. Sahs and Lesoing (1985) observed higher sweet corn yields in plots amended with beef feedlot manure than those that were inorganically fertilized during drought years. Heckman et al. (1987) found that field grown soybeans fertilized with sewage sludge had increased drought resistance and nitrogen fixation than the control treatment. Improved drought tolerance of crops grown in organically amended soils has been linked to the maintenance of optimum leaf health. In five-week old water stressed maize seedlings, Xu (2000) recorded higher photosynthetic rates when the soils were organically amended. HuiLan et al. (1998) noted that the application of organic amendments increased water stress resistance of sweet corn leaves. In particular, stomatal and cuticular conductances of the leaves were lower in these plants than in inorganically-fertilized plants.

An understanding of the processes that affect leaf photosynthesis - the primary determinant of yield - and how drought stress can impair the photosynthetic process is
essential to the elucidation of the mechanism of drought stress amelioration in crops grown in organically amended soils.

**Impact of Drought Stress on Photosynthetic Electron Transport**

During photosynthesis, green leaf tissues absorb solar energy and convert it into organic compounds. The process requires proper electron transfer throughout the thylakoid membrane. For every one photon of energy, two electrons from Photosystem II (PSII) oxidize water to oxygen, and reduce NADP$^+$ to NADPH via Fd-NADP reductase. ATP is also synthesized by the passage of H$^+$ from the lumen to the stroma (Figure 2.1). NADPH and ATP serve as reducing equivalents for the reduction of CO$_2$ to sugars in the stroma.

![Diagram of electron transport and proton movement](http://bioweb.wku.edu/courses/Biol120/images/z-scheme_grey.jpg)

**Figure 1.1.** The transfer of electrons and protons in the thylakoid membrane. Water is oxidized and protons are released in the lumen by Photosystem II. Photosystem I reduces NADP$^+$ to NADPH in the stroma. Protons transported into the lumen by the cytochrome $b_6$ complex contribute to the electrochemical proton gradient. These protons then diffuse to the ATP synthase enzyme where ATP is synthesized in the stroma. Both ATP and NADPH are used to form sugars in the dark reaction of the stroma. The solid line represents electron transfer. ©2005 from [http://bioweb.wku.edu/courses/Biol120/images/z-scheme_grey.jpg](http://bioweb.wku.edu/courses/Biol120/images/z-scheme_grey.jpg) by Robert Wyatt. Reproduced by permission of Robert Wyatt.

At the onset of moisture stress, stomatal openings close due to the decreased turgor pressure in the guard cells and/or the upregulation of abscisic acid (ABA) synthesis in the roots (Desikan et al., 2003). Stomatal closure is beneficial as it decreases transpiration and temporarily increases water use efficiency (Taiz and Zeiger, 1998). Prolonged closure, however, limits CO$_2$ influx and photochemical efficiency creating an over-reduced environment in the chloroplasts (Bray et al., 2002). Moisture stress during
high light conditions inevitably causes improper transfer of electrons to oxygen resulting in excessive reactive oxygen species (ROS) formation (Bray et al., 2002).

Reactive oxygen species are naturally generated due to the intrinsic inefficiencies of photosynthetic electron transport. Under saturating CO₂ conditions, 10-20% of electrons are still transferred to intracellular oxygen (Asada and Takahashi, 1987). Plants have evolved a scavenging mechanism to maintain steady-state levels of ROS as high concentrations can cause membrane lipid peroxidation, gene mutation and inactivation, and senescence (Asada, 1994).

**Formation of Reactive Oxygen Species**

Photosystem I (PSI) is the primary site of ROS formation (Mano, 2002). In the overreduced environment of the chloroplast, NADPH concentrations are much greater than NADP⁺. Newly accepted electrons by PSI, therefore, are less likely to be properly transferred to NADP⁺. Instead, the abundant intracellular oxygen found in the chloroplast serves as a reducible substrate. The reduction of oxygen to superoxide (O₂⁻) occurs via the Mehler reaction. Superoxide is a free radical that is highly reactive with the thiol and iron-sulfur clusters of peptides and DNA bases (Mano, 2002). Superoxide dismutase (SOD), an O₂⁻ scavenger, is contained in the chloroplast at a concentration of 50 μM (Asada, 1994). The enzyme is located adjacent to PSI on the stromal side of the thylakoid membrane or free floating in the stroma (Asada, 1994). Although total concentrations are low, the high affinity for O₂⁻ and the strategic location of the antioxidant renders great efficiency to reduce O₂⁻ to H₂O₂.

\[
2 \text{O}_2^- + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2
\]

Peroxide is not a free radical, *per se*, but it is highly water soluble and easily diffuses across membranes. Peroxide can serve as both a reductant or oxidant in forming other free radicals, the most dangerous of which is the hydroxyl radical (OH⁻) which is formed via the Haber-Weiss reaction (Perl-Treves and Perl, 2002).

\[
\begin{align*}
\text{H}_2\text{O}_2^+ \text{Fe}^{+2} & \rightarrow \text{OH}^- + \text{OH}^+ + \text{Fe}^{+3} \\
\text{O}_2^- + \text{Fe}^{+3} & \rightarrow \text{O}_2 + \text{Fe}^{+2} \\
\text{H}_2\text{O}_2^+ + \text{O}_2^- & \rightarrow \text{O}_2^- + \text{OH}^- + \text{OH}^-
\end{align*}
\]
The hydroxyl radical is extremely reactive, oxidizing everything in its vicinity as it has no specific targets (Mano, 2002). Hydroxyl destroys membrane integrity by producing lipid peroxides.

\[ \text{OH}^\cdot + \text{H from } -\text{CH}_2- \rightarrow \text{L}^\cdot \text{ (lipid radical)} \]

\[ \text{L}^\cdot + \text{O}_2 \rightarrow \text{LOO}^\cdot \text{ (lipid peroxyl radical)} \]

\[ \text{LOO}^\cdot + \text{O}_2 \rightarrow \text{LOOH} \text{ (lipid hydroperoxide)} \]

Due to its high toxicity, there are no antioxidants specific to scavenge OH^\cdot (Wolff et al., 1986). Instead, leaf health is protected by maintaining OH^\cdot substrates at low levels (Perl-Treves and Perl, 2002). Superoxide dismutase is arguably the most important first response antioxidant as it determines the concentration of \text{O}_2^\cdot \text{ and H}_2\text{O}_2 \text{ available for reaction (Perl-Treves and Perl, 2002).}

Peroxide is reduced to water by ascorbate peroxidase (APX). Total concentrations are low (70 µM), and the enzyme is strategically located on the stromal side of the thylakoid membrane or free floating in the stroma (Foyer, 2002). Ascorbate peroxidase reduces \text{H}_2\text{O}_2 \text{ in one of two reactions: the water-water cycle or the Halliwel-Asada cycle, also know as the ascorbate-glutathione cycle (Foyer, 2002). In the water-water cycle, electrons released by the oxidation of intracellular water are used to regenerate ascorbate (AsA), the reducing substrate that is catalyzed by thylakoid bound APX (tAPX) to reduce \text{H}_2\text{O}_2 \text{ to H}_2\text{O.}

\[ 2\text{AsA} + \text{H}_2\text{O}_2 \xrightarrow{\text{tAPX}} 2\text{MDA} + 2\text{H}_2\text{O} \]

\[ 2\text{MDA} + \text{Fd}_{\text{red}} \rightarrow \text{AsA} + \text{Fd} \]

where MDA= monodehydroascorbate, Fd=ferredoxin, Fd_{\text{red}}=reduced ferredoxin

In the ascorbate-glutathione cycle, stromal APX (sAPX) catalyzes the reduction of \text{H}_2\text{O}_2 \text{ to H}_2\text{O using AsA and glutathione (GSH) as reducing substrates.}

\[ 2\text{AsA} + 2\text{H}_2\text{O}_2 \xrightarrow{\text{sAPX}} 2\text{MDA} + 2\text{H}_2\text{O} \]
2MDA + NADP $\rightarrow$ 2 AsA + NADP$^+$

OR

2MDA + DHA + 2GSH $\rightarrow$ AsA + GSSH

GSSH + NADPH + H$^+$ $\rightarrow$ 2GSH + NADP$^+$

Where MDA = monodehydroascorbate, MDAR = monodehydroascorbate reductase, DHA = dehydroascorbate, GSH = glutathione, GSSG = oxidized glutathione

Both cycles are advantageous as electrons are used directly from the photosynthetic apparatus (reduced Fd and NADPH) as reducing power. This not only scavenges ROS produced in the environment, but removes the factors that created conditions conducive for ROS formation (Mittler, 2002). Figure 2.2 provides a comprehensive view of the thylakoid antioxidants involved in ROS quenching.

Another antioxidant that scavenges H$_2$O$_2$ is catalase (CAT). Located exclusively in the peroxisome, CAT is found in high concentrations (~4mM), some of which is in...
crystalline form (Asada, 1994). Although it requires no reducing equivalents, CAT is only stimulated by mM concentrations of \( \text{H}_2\text{O}_2 \) making it less responsive than APX to oxidative stress (Asada, 1994).

\[
2 \text{H}_2\text{O}_2 \xrightarrow{\text{CAT}} 2 \text{H}_2\text{O} + \text{O}_2
\]

Tolbert et al. (1969) conducted a survey of leaf peroxisome activity in 10 plant species and observed that in C\(_3\) plants, the oxidation of glycolate, a product of photorespiration, accounted for most of the \( \text{H}_2\text{O}_2 \) scavenged by CAT. Lyu-bimov and Zastrizhnaya (1992) observed that CAT activity in C\(_4\) plants was not driven by the production of glycolate, but by the formation of \( \text{H}_2\text{O}_2 \) from SOD activities.

**Humic Substances May Ameliorate Crop Drought Stress**

There are a few studies that document increased drought tolerance of creeping bentgrass (*Agrostis stolonifera*) (Waddington et al., 1978), tall fescue (*Festuca arundinacea*) (Zhang et al., 2005), soybean (*Glycine max*) (Heckman et al., 1987), and corn (*Zea mays*) (Sahs and Lesoing, 1985; Hornick, 1988; HuiLan et al., 1998; Mamo et al., 2000; Xu, 2000) in compost amended soils. Most interesting are the improvements in crop drought stress tolerance when there where no apparent differences in soil water holding capacity among treatments (HuiLan et al., 1998; Mamo et al., 2000; Xu, 2000; Zhang et al., 2005).

Researchers speculate that the hormone-like properties of humic substances may play a causal role in drought stress amelioration (Serdyuk et al., 1999; Kulikova et al., 2003; Chen et al., 2004; Quaggiotti et al., 2004; Zhang and Ervin, 2004). Frakenberger and Arshad (1995) summarized research that documented the alteration of plant development by external hormones. Zhang et al. (2005) applied nine differently processed biosolids based on agronomic nitrogen needs to tall fescue planted in calcined clay and observed increased SOD among the drought stress treatments. Interestingly, the plants with the greatest drought tolerance (i.e., greatest SOD activity) were planted in biosolids treatments that contained the greatest auxin content. Zhang and Ervin (2004) foliarly applied humic acid derived from leonardite to two mature field grown bentgrass cultivars. The researchers observed that unashed samples of the compound contained the cytokinin sugar, zeatin riboside, and that the application of this material increased the
zeatin riboside content of both cultivars. An increase in cytokinin has been linked to increased antioxidant activity and drought tolerance (Musgrave, 1994).

Other studies have documented improved crop drought stress tolerance following foliar applications of humic substances (Mladenova et al., 1998). Xudan (1986) foliarly applied fulvic acid to pot grown wheat (*Triticum aestivum*) plants prior to imposing a nine-day dry down period. These plants maintained greater stomatal conductances, contained greater chlorophyll contents and increased $^{32}$P uptake relative to the control. Yan and Schmidt (1993) applied a commercially available seaweed extract to pot grown drought stressed perennial ryegrass and observed increased cell membrane fluidity and permeability relative to the control treatment. Zhang and Schmidt (1999, 2000) foliarly applied a commercially available seaweed extract and humic acid solution to drought-stressed tall fescue, creeping bentgrass, and Kentucky bluegrass (*Poa pratensis*), and observed an increase in leaf water status and antioxidant activities relative to the control.

In the preceding experiments, humic substances were foliarly applied to the crop. Research exploring possible ameliorative effects of land applied compost humic substances on drought stressed agronomic crops is lacking. Further investigation is required to discover whether organic matter fractions in compost may elicit plant physiological benefits under field conditions.

**Summary and Conclusions**

Composted organic amendments are frequently applied to land as a means of environmentally sound waste management. Their application to land is often based on estimated nitrogen mineralization rates for the materials. The use of composts have consistently shown to be a viable means of improving soil quality parameters including soil water holding capacity, organic and humified carbon contents, and soil macro- and micronutrient status. Researchers have observed that the use of composted organic amendments may improve plant health beyond the fertility value. Of particular interest is the apparent ameliorating effect of organic amendments on drought-stressed crops.

Researchers speculate that the hormonal properties of humic substances within composts play a causal role in ameliorating drought stress. Humic substances are biologically active compounds formed during the composting process that are resistant to microbial degradation.
Most experiments exploring the potential ameliorating effects of humic substances on crop growth are carried out in pot studies where humified materials are foliarly applied to seedlings. Research exploring possible ameliorative effects of land applied compost humic substances on drought stressed agronomic crops is lacking. Further investigation is required to discover whether organic matter fractions in compost may elicit plant physiological benefits under field conditions.

References


due to organic waste applications: a review. J. Environ. Qual. 10:133-141.


